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Protective effects of Brussels sprouts, oligosaccharides and fermented milk towards 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced genotoxicity in the human flora associated F344 rat: role of xenobiotic metabolising enzymes and intestinal microflora

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Abstract

We investigated the chemoprotective effects of four common constituents of the human diet, i.e. a fermented milk, inulin, oligofructose and Brussels sprouts, towards 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced genotoxicity in male Fischer 344 rats harbouring a human intestinal microflora. We found that the four dietary components significantly reduced IQ-induced DNA damage in hepatocytes (reduction ranged from 74% with inulin to 39% with Brussels sprouts) and colonocytes (reduction ranged from 68% with inulin to 56% with Brussels sprouts). This chemoprotective effect correlated with the induction of hepatic UDP-glucuronosyl transferase following Brussels sprouts consumption, and with alterations of bacterial metabolism in the distal gut (acidification, increase of butyrate proportion, decrease of β-glucuronidase activity) following inulin consumption.

Keywords: *Brassica*; Fermented milk; Genotoxicity; 2-Amino-3-methylimidazo[4,5-*f*]quinoline; Oligosaccharides

1. Introduction

Dietary epidemiological studies implicate heterocyclic amines (HAs), mutagenic/carcinogenic compounds formed from high-protein diets during cooking, as risk factors in the aetiology of human colon and breast cancer [1]. During the last decades strong efforts have been made to identify dietary constituents which protect against the genotoxic and carcinogenic effects of HAs. More than 600 complex mixtures and individual compounds contained in the human diet have been studied for chemoprotective effects towards HAs [2]. Among them, *Brassica* vegetables, dietary fibres and milk products containing lactic acid bacteria, or their individual constituents, have been shown to be protective [2,3]. However, most of the studies referred above have been performed in vitro.

The aim of the present study was to investigate the potential protective effects of three types of dietary products, i.e. fermented milks, oligosaccharides and *Brassica* vegetables, towards 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced genotoxic effects in vivo, in liver and colon cells of human flora associated rats. IQ is a potent rodent carcinogen and liver and colon are the main target organs for tumour formation. We recently found that the origin of the intestinal flora, rat or human flora, has a strong impact on the genotoxic effect of IQ [4]. Therefore, in the present study, we decided to use human flora associated (HFA) rats which reflect the situation in humans better than conventional rats [5]. Many of the studies investigating dietary protection towards HAs were carried out with bacterial indicators of

Abbreviations: BCFA, branched chain fatty acids; CYP450, cytochrome P450; FU, fluorescence units; GST, glutathione-*S*-transferase; HAs, heterocyclic amines; HFA, human flora associated; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; NAT, *N*-acetyl transferase; SCFA, short chain fatty acids; SCGE, single cell gel electrophoresis; UDPGT, UDP-glucuronosyl transferase; XME, xenobiotic metabolising enzymes

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genotoxicity. However, as we reported earlier [2], bacterial assays do not reflect the complex metabolism of HAs and, therefore, may give misleading results. As an experimental model, we used the single cell gel electrophoresis (SCGE) assay, which enables the detection of chemoprotective effects in organs which are targets of tumour formation by HAs.

As a representative fermented milk, we used a commercial product containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus casei*, that has been shown to modify the composition and metabolism of the intestinal microflora [6]. The current state of knowledge on the effects of fermented foods' bacteria towards HAs is reviewed in the article of Knasmüller et al. [7]; they conclude that the most important detoxification mechanism would be a direct binding of HAs to the cell wall of certain bacterial strains contained in fermented foods. As examples of oligosaccharides, we used two compounds, an oligofructose with a chain length of 2–8 sugar molecules, and inulin, a polyfructose with a chain length of 2–60 sugar molecules. Both compounds are commonly added to foods as prebiotics [8]. It has been shown earlier that dietary fibres which are neither digested nor fermented bind to HAs [9] and that fibre-rich plant preparations prevent the formation of IQ-induced preneoplastic lesions in the colon [2]. However, oligofructoses, which are extensively fermented in the colon by intestinal bacteria, have never been investigated for such protective effects. As a representative *Brassica* vegetable, we used Brussels sprouts. It is known that this vegetable is rich in glucosinolates which, upon hydrolysis by either plant or bacterial myrosinases (E.C. 3.2.3.1.), generate several bioactive compounds, including isothiocyanates; these derivatives are believed to be the main responsible agents for the chemoprotective effects of *Brassica* vegetables [10,11]. We recently demonstrated that garden cress (*Lepidium sativum*) inhibits IQ-induced genotoxic and preneoplastic lesions; this effect was correlated to an induction of the activity of hepatic UDP-glucuronosyl transferase (UDPGT), an enzyme known to detoxify HAs [12,13].

As an attempt to understand the mechanisms underlying the potential chemoprotective effects of the Brussels sprouts, the oligosaccharides and the fermented milk, we also monitored the impact of these compounds on a panel of xenobiotic metabolising enzymes (XME) which play a role in the intestinal metabolism of HAs, i.e. hepatic total cytochrome P450 (CYP450 E.C. 1.14.14.1), cytochrome P450 1A (CYP 1A), glutathione-*S*-transferase (GST E.C. 2.5.1.18), *N*-acetyl transferase (NAT E.C. 2.3.1.5) and UDPGT (E.C. 2.4.7.17). We also measured the influence of the different diets on the caecal pH, on bacterial metabolites including short chain fatty acids (SCFA), lactic acid and ammonia, and on the bacterial enzyme β-glucuronidase (E.C. 3.2.1.31). Indeed several findings indicate that β-glucuronidase hydrolyses HA-glucuronide conjugates in the colon and it has been postulated that the cleavage products may undergo enterohepatic circulation and/or be activated to DNA-reactive metabolites in the colon and other organs [7].

2. Experimental

2.1. Chemicals

IQ was obtained from Toronto Research Chemicals Inc. (Toronto, Canada). Inorganic salts for SCGE assays, ethidium bromide and acetonitrile came from Merck (Nogent-sur-Marne, France). Collagenase, proteinase K, William's medium E, normal and low melting agarose and RPMI were obtained from Gibco (Paisley, UK). All other chemicals came from Sigma-Aldrich (St. Quentin Fallavier, France).

2.2. Diets

The diets were semi-synthetic diets designed to match a normal human-type diet while being compatible with the rodent digestive physiology. Therefore, lipids and proteins of both animal and vegetable origins, as well as cellulose and cooked starch, were included. The control diet consisted of 28.985% (w/w) cornstarch, 29% mashed potatoes, 5% saccharose, 5% casein, 12% soybean isolate, 3% corn oil, 3% lard, 0.015% cholesterol, 6% cellulose, 8% minerals and vitamin mixture [14]. In the oligofructose and inulin diets, saccharose and 5% cornstarch were replaced by either 10% oligofructose (RAFTILOSE® P95, Orafti, Tienen, Belgium) or 10% inulin (RAFTILOSE® Synergy 1, Orafti). In the Brussels sprouts diet, 4% cornstarch, 4% mashed potatoes and 2% soybean isolate were replaced by 10% freeze-dried Brussels sprouts containing 23 μmol glucosinolates/g dry material. Brussels sprouts are usually cooked before consumption; therefore, sprouts were heated before incorporation into the diet [15]. This treatment inactivates plant myrosinase so that glucosinolates are expected to reach the colon and to be hydrolysed by bacterial myrosinase [16]. The fermented milk diet was a control diet supplemented with a commercial fermented milk containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus casei* (1 ml fermented milk for 1 g of dietary powder). Diets were prepared freshly every 2 days by mixing thoroughly 50 g powder with 100 ml of water (control, inulin, oligofructose, Brussels sprouts diets) or with 50 ml of fermented milk and 50 ml of water (fermented milk diet). All dietary powders were packed in double-vacuum bags and sterilised by γ irradiation at 45 kGy (UAR, Villemoisson-sur-Orge, France).

2.3. Animals

Animals were germ-free male Fischer 344 rats provided by the breeding facility of Unité d'Ecologie et de Physiologie du Système Digestif (INRA, Jouy-en-Josas, France). Throughout the study, they were kept in a room that was

maintained at constant temperature and humidity (21 ± 1 °C, 50 ± 5%) with a 12 h light/dark cycle. They were housed (three to four rats/cage) in Trexler type isolators fitted with a rapid transfer system (La Calhène, Vélizy, France) and were given free access to their diet and to sterilized tap water.

2.4. Experimental design

All procedures were carried out in accordance with the European guidelines for the care and use of laboratory animals.

To obtain human flora associated rats, initially germ free rats were inoculated orally at 3 months of age with a whole faecal flora collected from a healthy adult man as described previously [14]. HFA rats were then adapted to the flora and to the control diet for 1 week.

Two independent experiments were subsequently carried out. In the first one, 23 HFA rats were randomly allocated to five dietary groups (four animals for each experimental diet and seven animals for the control diet). After 4 weeks, animals were gavaged with IQ suspended in 0.2 ml corn oil (90 mg/kg). Four hours later, animals were killed by CO_2 inhalation and the liver and the colon were collected for SCGE assay [4]. Three rats from the group receiving the control diet were used as a negative control and received corn oil only. Liver and colon cells were isolated as described by Bradley et al. [17] and Brendler et al. [18], respectively. Isolated cells were treated as described by Kassie et al. [4].

In the second experiment, 16 HFA rats were fed as described above for 4 weeks but only the control, Brussels sprouts, inulin and fermented milk diets were used; each dietary group consisted of four animals. During the last week of the experiment, faeces from each rat were collected for the measurement of β-glucuronidase activity. At the end of the experiment, animals were killed by CO_2 inhalation and livers were collected, perfused with KCl (150 mM) and stored at −80 °C until preparation of subcellular fractions for XME assays. The caecal pH was measured and caecal contents were distributed into several vials stored at −80 °C for SCFA, lactic acid and ammonia analyses.

2.5. Analysis of xenobiotic metabolising enzymes

Hepatic microsomes and cytosols were prepared as described by Ryan et al. [19] and stored in aliquots at −80 °C until analysis. All enzyme assays were performed in duplicate. Protein concentrations were determined according to the method of Lowry et al., using bovine albumin as a standard [20]. The concentration of CYP450 was assayed in liver microsomes according to the method of Omura and Sato [21]. The ethoxyresorufin activity, representing CYP 1A, was measured according to Lubet et al. [22]. UDPGT activities were measured in the microsomes with two substrates: chloramphenicol was used to measure predominantly the UDPGT-form 2 by the radiometric method of Young and Lietman [23], with slight modifications [14]; 4-methylumbelliferone was used to analyse preferentially the UDPGT-form 1 as described by Lilienblum et al. [24]. The activity of cytosolic GST was determined spectrophotometrically (λ = 340 nm) at room temperature using 1-chloro-2,4-dinitrobenzene as a substrate, following the method of Habig et al. [25] miniaturized to a microplate (Spectrafluor plus Tecan, Trappes, France). NAT activity was determined according to the procedure described by Grant et al. [26], with slight modifications to adapt the method for rat hepatic tissues. Briefly, the reaction mixture contained 100 μM of aminofluorene (substrate), 100 μM of acetyl-CoA (cofactor) and 50 μg of cytosolic proteins. After 3 and 6 min incubation at 37 °C, the reaction was stopped by adding 100 μl acetonitrile to precipitate proteins; samples were then frozen at −20 °C until analysis. After thawing and centrifugation at 5000 × *g* for 2 min, 50 μl of the supernatants were injected to HPLC with an autosampler (Beckman 507e, Villepinte, France) onto a reversed phase column packed with LiChrospher® 100 RP-18 (5 μm, 25 cm) (Merck). Samples were eluted at 2.0 ml min^{-1} with a mobile phase consisting of 20 mM $NaClO_4$ (pH 2.5) and acetonitrile (50% (v/v)). Compounds were detected by UV absorbance at 270 nm. Under these conditions, the retention times of aminofluorene and its metabolite, acetamidofluorene, were 2.12 and 3.80 min, respectively.

2.6. Analysis of fermentation and β-glucuronidase activity in caecal and faecal contents

All analyses were performed in duplicate. β-Glucuronidase was measured spectrophotometrically by the rate of release of *p*-nitrophenol (λ = 400 nm) from the *p*-nitrophenylglucuronide as described by Andrieux et al. [27]. Enzymatic activity was expressed as micromole (μmol) of product formed per minute (min) and per gram (g) of wet faeces. SCFA were analysed after water extraction of acidified samples, using a gas–liquid chromatograph (Nelson 1020, Perkin-Elmer, St. Quentin en Yvelines, France) equipped with a flame-ionisation detector and a wide-bore column (15 m × 0.53 mm) (FSCAP Nukol, Supelco, St. Quentin Fallavier, France) impregnated with SP 1000. Carrier gas (He) flow-rate was 10 ml min^{-1}, inlet temperature 100 °C, detector temperature 280 °C. 2-(Ethyl)butyrate was used as the internal standard [27]. Lactic acid was determined enzymatically [28] and ammonia was analysed using the Berthelot method adapted by Dropsy and Boy [29]. Lactic acid and ammonia were measured using a continuous Technicon Autoanalyser®.

2.7. Single cell gel electrophoresis (SCGE) assay

Phosphate buffered saline, alkali (electrolysis) buffer, lysis solution, neutralization buffer and ethidium bromide stain were prepared as described by Singh et al. [30]. Agarose coated slides were made with 1.5% normal melting agarose according to the protocol of Klaude et al. [31].

Microgel electrophoresis was performed according to Singh et al. [32]. Briefly, 10 000 cells suspended in 90 μl 0.5% low melting point agarose were transferred to a slide pre-coated with normal melting agarose and covered with a cover slip. After allowing the low melting agarose to solidify by putting the slide on a cooled metal plate for 2 min, the cover slip was carefully removed and the slides submersed into lysis solution for 24 h. Subsequent to alkali treatment (pH 13.00, 20 min) and electrophoresis (300 mA, 25 mV, 20 min; Biometra Standard Power Pack P25), the slides were removed from the electrophoresis chamber, washed two times with neutralization buffer and stained with ethidium bromide. Analysis of DNA damage was made by measuring the comet tail lengths of the indicator cells with a fluorescence microscope (Nikon, EFD-3, 125-fold magnification) connected to a monitor with a specific macro for the NIH-public domain image analysis programme [33]. From each organ, three slides were prepared, and from each slide, 50 cells were analysed.

2.8. Statistical analyses

The results were expressed as mean ± S.E.M. ($n = 3$ for SCGE assay and $n = 4$ for all other assays).

In SCGE assay, we ascertained the genotoxicity of IQ by comparing rats fed on the control diet and gavaged with IQ with rats fed on the control diet and gavaged with corn oil; comparison was performed using the Student's t test.

The effect of experimental diets on IQ-genotoxicity, hepatic XME, fermentation and bacterial β-glucuronidase activity was analysed using ANOVA (Statview®, Abacus concept). When ANOVA indicated significant differences, groups receiving the experimental diets were compared with the group receiving the control diet using the Dunnett's test. Statistical significance was accepted at the $P < 0.05$ level.

3. Results

3.1. IQ-induced DNA damage

As expected, IQ-induced DNA damage in the group receiving the control diet and gavaged with corn oil (negative control) tail lengths were 7.2 ± 0.7 μm and 5.9 ± 0.3 μm, in the colon and in the liver, respectively; the data were 10-fold increased in the colon (79.0 ± 13.8 μm) and in the liver (64.8 ± 6.9 μm) of HFA rats receiving the control diet and gavaged with IQ.

3.2. Effect of the experimental diets on the IQ-induced DNA damage

The results of the SCGE assays on colonocytes and hepatocytes are shown in Fig. 1. In the liver, cells obtained from HFA rats fed on the different experimental diets and gavaged with IQ exhibited significantly lower DNA damages than cells from rats fed on the control diet and gavaged with IQ: the reduction ranged from 74% with inulin to 39% with Brussels sprouts. In the colonocytes, the comet tail lengths were reduced by 70% with inulin and fermented milk diets, and by 55% with Brussels sprouts and oligofructose diets when compared with the control group ($P < 0.05$).

3.3. Effect of the experimental diets on hepatic xenobiotic metabolising enzymes

The results of XME measurements are shown in Table 1. The consumption of Brussels sprouts for 4 weeks significantly increased the activity of UDGPT-form 1 (164% of the control value) while it did not modify the other XME. Inulin and fermented milk were inefficient to modify total CYP450 concentration as well as CYP1A, GST, NAT, UDPGT-form 1 and UDPGT-form 2 activities.

3.4. Effect of the experimental diets on faecal β-glucuronidase activity

Inulin consumption significantly decreased the activity of β-glucuronidase by 50% (Fig. 2) while the Brussels sprouts and fermented milk diets were inefficient.

3.5. Effect of the diets on the caecal pH and on fermentation metabolites

Caecal pH did not change in rats fed on the Brussels sprouts or fermented milk diets but decreased significantly with inulin (Table 2). The total SCFA concentration was not influenced by any of the experimental diets but the SCFA profiles differed significantly from those observed in the control animals. Brussels sprouts and fermented milk increased the proportion of acetate and propionate, respectively (106 and 115% of the control values); consumption of inulin doubled the proportion of butyrate at the expense of acetate, propionate and branched chain fatty acids (respectively 88, 69 and 50% of the control values). Lactic acid and ammonia concentrations were identical in all groups.

4. Discussion

We showed that the different dietary regimens, i.e. the fermented milk containing lactic acid bacteria, the oligosaccharides inulin and oligofructose, and the cooked Brussels sprouts reduced the induction of DNA damage caused by the heterocyclic amine IQ in liver and colon cells of human flora associated rats. Furthermore, the results suggest that different mechanisms may account for the chemoprotective effects of these dietary constituents.

The protective effect of the fermented milk containing a mixture of three lactic acid bacteria, including those commonly found in yoghurts, was not unexpected, as two earlier in vivo studies indicated that lactic acid bacteria are able

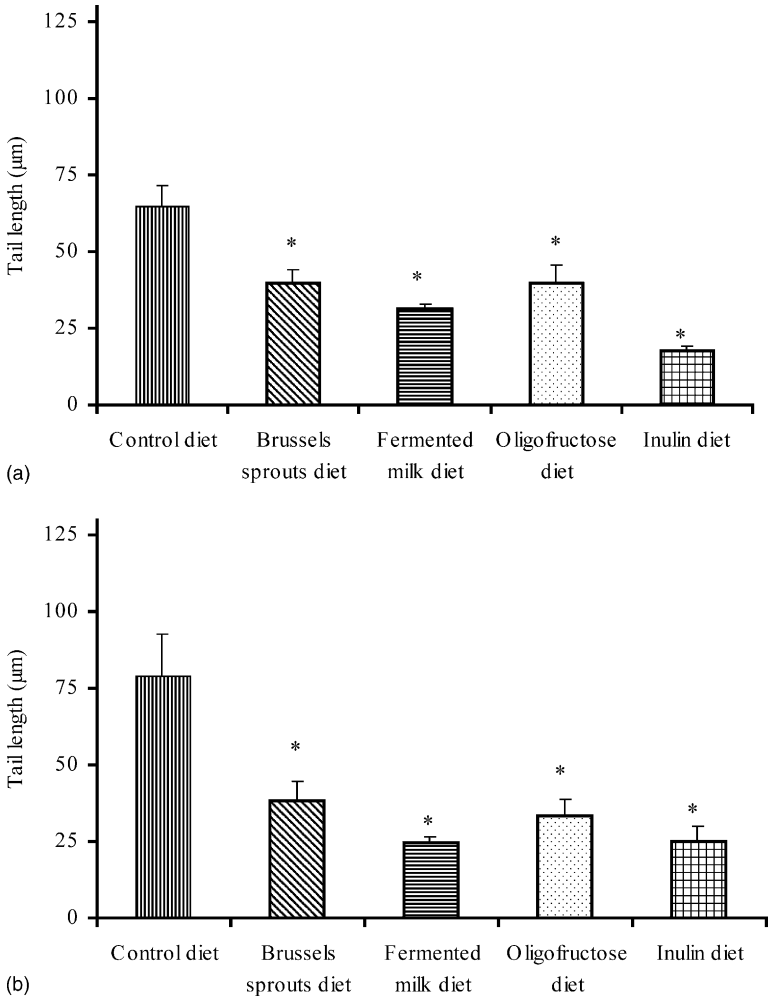

Fig. 1. DNA damage in hepatocytes (a) and colonocytes (b) of HFA rats gavaged with IQ and fed on different experimental diets. Four hours after IQ treatment, animals were killed and cells isolated from liver and colon were submitted to SCGE assay. Bars indicate means ± S.E.M. of comet tail lengths measured with 9–12 slides (three to four animals per treatment group, three slides per organ, 50 cells per slides). Asterisks indicate significant differences as compared with the group fed on the control diet ($P < 0.05$).

to reduce the genotoxic effects of HAs [34], [35]. However, in both studies, bacteria in the fermented milk were freeze-dried. Recently, Tavan et al. [36] carried out a study with a fermented milk containing *Bifidobacterium longum* and *Streptococcus thermophilus*; they found a reduction in the formation of colonic aberrant crypt foci caused by a mixture of three HAs but the results of SCGE assays were inconclusive. Therefore, to our knowledge, our report is the

Table 1
Effect of different diets on hepatic xenobiotic metabolising enzymes in human flora associated rats

Enzyme	Control diet			Brussels sprouts diet			Inulin diet			Fermented milk diet		
	Mean	S.E.M.	%	Mean	S.E.M.	%	Mean	S.E.M.	%	Mean	S.E.M.	%
CYP 450 (nmol/mg protein)	0.76	0.03	100	0.77	0.01	101	0.84	0.02	111	0.83	0.04	109
CYP 1A (FU[a]/min/mg protein)	4.9	0.3	100	6.0	0.4	122	5.6	0.2	114	5.3	0.2	108
GST (nmol/min/mg protein)	1158	112	100	1612	74	139	1131	45	98	1044	25	90
NAT (nmol/min/mg protein)	3.0	0.1	100	2.5	0.1	84	2.8	0.1	94	3.1	0.1	104
UDPGT-form 1 (nmol/min/mg protein)	514	18	100	841[b]	69	164	483	12	94	539	41	105
UDPGT-form 2 (nmol/min/mg protein)	1.1	0.2	100	1.3	0.1	118	1.2	0.1	109	1.1	0.1	100

Animals ($n = 4$) were fed on the experimental diets (control, Brussels sprouts, inulin and fermented milk diets) for 4 weeks. After killing, microsomes and cytosols were prepared from livers as described in Section 2.5 and all analyses were made in duplicate.

[a] FU: fluorescence units.

[b] Significantly different from the control group (Dunnett's test, $P < 0.05$).

Table 2
Effect of different diets on the pH and on the concentration of fermentative metabolites in the caecum of human flora associated rats

	Control diet			Brussels sprouts diet			Inulin diet			Fermented milk diet		
	Mean	S.E.M.	%	Mean	S.E.M.	%	Mean	S.E.M.	%	Mean	S.E.M.	%
pH	6.3	0.2	100	6.3	0.2	100	5.8[a]	0.3	92	6.6	0.1	105
Total SCFA (μmol/g)	79	3	100	70	3	88	67	3	84	91	5	115
μmol/100 μmol SCFA												
Acetate	69	0	100	73[a]	0	106	61[a]	0	88	68	1	099
Propionate	13	0	100	11	0	85	9[a]	0	69	15[a]	0	115
Butyrate	14	0	100	12	0	86	27[a]	0	193	13	0	93
Valerate + caproate	2	0	100	2	0	100	2	0	100	2	0	100
BCFA	2	0	100	2	0	100	1[a]	0	50	2	0	100
Lactic acid (μmol/g)	3.4	0.5	100	3.6	0.5	106	2.6	1.1	76	5.0	1.1	147
Ammonia (μmol/g)	2.1	0.7	100	1.5	0.2	71	1.6	0.6	76	2.4	0.7	114

Animals were submitted for four weeks to experimental diets (Control, Brussels sprouts, Inulin and Fermented milk diets). After killing, caecal pH was measured, and caecal contents were analysed for SCFA, lactic acid and ammonia as described in the Section 2.6. Results are expressed by g of wet weight. All analyses were made in duplicate.

[a] Significantly different from the control group (Dunnett's test, $P < 0.05$).

first to clearly show that a fermented milk containing living bacteria protects against DNA damaging effects of IQ in vivo. Furthermore, we show that this protection takes place both in the liver and in the colon. The fermented milk we used had neither a significant impact on the activity of XME nor on the intestinal microbial metabolism (see Tables 1 and 2). Therefore, direct binding of IQ which has been described in several articles [7] may account for the protective effect.

The two oligosaccharides inulin and oligofructose also caused a significant reduction of DNA damage. Whereas the chemoprotective effect of inulin was pronounced (IQ-induced DNA damage was reduced by 74 and 68%, respectively, in the liver and in the colon), oligofructose was less effective in both organs. All earlier works on the protective effects of oligosaccharides were carried out using azoxymethane and dimethylhydrazine as model carcinogens [37–40] and, to our knowledge, this is the first report showing a chemoprotective effect of this kind of compounds towards a dietary heterocyclic amine. Although several findings have shown that dietary fibres of different origins bind IQ and other HAs [41–44] and that the frequency of IQ-induced aberrant crypt foci is reduced by diets enriched with wheat bran and fibres from cork and potato cell walls [45], it is unlikely that direct binding of IQ could be responsible for the protective properties of the oligosaccharides used in the present experiment. Indeed it is known that inulin, as well as oligofructose, are extensively fermented in the caecal content [46,47]. The most plausible explanation would be the reduction of β-glucuronidase activity. This hypothesis is supported by reports of Reddy [39] and Rowland et al. [38], in which a reduction of β-glucuronidase activity is paralleled by a decrease in the frequency of azoxymethane-induced colonic aberrant crypt foci. It has been hypothesized that hydrolysis of glucuronic acid-HA conjugates by β-glucuronidase leads to the release of DNA-reactive metabolites in the colon; these metabolites can also reach the liver via the enterohepatic circulation and cause DNA damage there [48,49]. On the other hand, we observed in the present study that the anaerobic breakdown of inulin dramatically increased the proportion of butyrate in the caecal content. In in vitro experiments, butyrate has been found to cause apoptosis of cancerous colon cells [50]; however, this mechanism does not seem relevant in our study since our experimental conditions were designed to reproduce solely the first step of the cancer process, i.e. DNA damage.

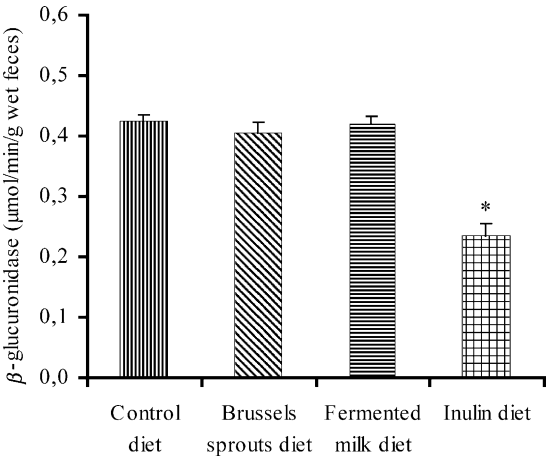

Fig. 2. β-Glucuronidase activity in the faeces of HFA rats fed on experimental diets for 4 weeks. Enzyme activity was determined by using *p*-nitrophenylglucuronide as a substrate. All measurements were made in duplicate. Bars indicate means ± S.E.M. of values obtained from four rats. Asterisks indicate significant differences compared with the group fed on the control diet ($P < 0.05$).

The Brussels sprouts-enriched diet was also protective, reducing IQ-induced DNA damage by approximately 50% in both liver and colon. We recently observed that garden cress (*Lepidium sativum*) juice prevents IQ-induced DNA damage and colonic preneoplastic lesions and this effect was paralleled by an increase of UDPGT activity [12,13]. Since conjugation with glucuronic acid is the main detoxification

pathway of HAs [51–54], we postulate that the increase of UDPGT activity occurring in the group receiving the Brussels sprouts diet may account for the protective effect of this *Brassica* vegetable. Both Brussels sprouts and garden cress contain glucosinolates which, upon hydrolysis by myrosinase, give rise mainly to isothiocyanates. These compounds are well known for their antimutagenic and anticarcinogenic effects (for review see Verhoven et al.) [10,11,16].

In conclusion, our findings show that common dietary constituents such as Brussels sprouts, oligosaccharides and fermented milks may protect against the carcinogenic effects of IQ and other structurally related HAs. Furthermore, they suggest that this protection involves interplay of various endogenous and bacterial metabolising enzymes.

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